INTEGRATED FILTRATION AND DETECTION DEVICE

Related Applications

This application claims the benefit of priority of U.S. Provisional Application Serial No. 60/272,233, filed February 28, 2001, the disclosure of which is incorporated herein by reference in its entirety.

Field of the Invention

The present invention relates to detection devices, and, more particularly, to devices for detecting microbial growth in a specimen.

Background of the Invention

It is often desirable or necessary to test fluid samples, such as liquid specimens, for contamination by live microorganisms. The presence of microbial contamination in clinical specimens is conventionally determined by culturing the specimens in the presence of nutrients and detecting microbial activity through changes in the specimen or the atmosphere over the specimen after a period of time.

It is often desirable to be able to test a specimen having a relatively large volume. Furthermore, it may be advantageous to increase the concentration of microorganisms in the specimen undergoing analysis so that changes in the specimen or adjacent atmosphere are intensified and thereby more easily and accurately detectable.

Summary of the Invention

According to certain embodiments of the present invention, an integrated filtration and detection device for collecting and detecting the growth of

microorganisms in a specimen includes a container defining a chamber therein. The container has an inlet and an outlet in fluid communication with the chamber. A filter is mounted in the chamber between the inlet and the outlet. A sensor is mounted in the chamber. The sensor is operative to exhibit a change in a measurable property thereof upon exposure to changes in the chamber due to microbial growth.

According to certain method embodiments of the present invention, a method for collecting and detecting the growth of microorganisms in a specimen includes providing an integrated filtration and detection device. The integrated filtration and detection device includes a container defining a chamber therein and having an inlet and an outlet in fluid communication with the chamber. A filter is mounted in the chamber between the inlet and the outlet. A sensor is mounted in the chamber and is operative to exhibit a change in a measurable property thereof upon exposure to changes in the chamber due to microbial growth. The specimen is passed into the chamber through the inlet, through the filter and out of the chamber through the outlet to collect the microorganisms on the filter. The measurable property of the sensor is detected.

Objects of the present invention will be appreciated by those of ordinary skill in the art from a reading of the figures and the detailed description of the preferred embodiments which follow, such description being merely illustrative of the present invention.

Brief Description of the Drawings

The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate embodiments of the invention and, together with the description, serve to explain principles of the invention.

Figure 1 is a perspective view of an integrated filtration and detection device according to embodiments of the present invention;

Figure 2 is a cross-sectional view of the device of **Figure 1** taken along the line **2-2** of **Figure 1**;

Figure 3 is a side elevational view of the device of **Figure 1**, illustrated with an associated specimen supply, an associated waste receptacle and an associated pump according to embodiments of the present invention;

Figure 4 is a side elevational view of the device, waste receptacle and pump of **Figure 3**, illustrated with an associated wash fluid supply according to embodiments of the present invention;

Figure 5 is a side-elevational view of the device of **Figure 1**, illustrated with an associated culturing medium supply according to embodiments of the present invention;

Figure 6 is a side-elevational view of a sample assembly including the device of **Figure 1**, along with an associated length of tubing and a clamp, wherein the device contains the culturing medium according to embodiments of the present invention;

Figure 7 is a fragmentary, cross-sectional view of a measuring apparatus and the sample assembly of **Figure 6** mounted therein;

Figure 8 is a block diagram representing a method of collecting and detecting the growth of microorganisms in a specimen according to embodiments of the present invention; and

Figure 9 is a cross-sectional view of an integrated filtration and detection device according to further embodiments of the present invention.

Detailed Description of Embodiments of the Invention

The present invention now will be described more fully hereinafter with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. Like numbers refer to like elements throughout. In the figures, layers, components or regions may be exaggerated for clarity.

The devices and methods of the present invention may be used to detect the presence of microorganisms in clinical specimens, such as blood or other body fluids, and in non-clinical specimens such as food, juices, cosmetics, shampoos, pharmaceuticals or consumer products, by culturing the specimens with a growth or culturing medium in a container of a device according to the invention. The specimen may be filtered through a filter disposed in the container to capture a sample including microorganisms from the specimen. The presence and the identification of microorganisms may be determined by, for example, detecting or measuring changes in the pH of the specimen or the production of CO_2 within the specimen using a sensor disposed in the container. Thus, the devices and methods may provide a non-invasive means for detecting the presence of microorganisms in specimens by measuring an increase in metabolic byproducts produced by the microorganisms (directly or indirectly). Moreover, the culturing medium and the filter may be sterilized and maintained sterile. Further, the container may be effectively sealed and the integrity of the seal maintained during the evaluation process.

With reference to **Figures 1** and **2**, an integrated filtration and detection device according to the present invention is shown therein and generally designated by reference number **100**. The device **100** may be used to filter a sample of microorganisms from a volume of a specimen selected for analysis and also to detect the growth of the microorganisms without directly handling the filter or exposing the filter, the specimen or the sample to contamination. Furthermore, the device **100** is adapted for convenient and effective use with electronic and automated measuring apparatus reducing labor costs and the risk of inadvertent process-induced contamination. The device **100** may be sealable and sterilizable. Moreover, the device **100** may be disposable after a single use.

Turning to the construction of the device **100** in more detail, the device **100** includes a container **110**. The container **110** may be formed of an economic or inexpensive material. Preferably, the container **110** is formed of a material which

may be effectively sterilized and sealed using conventional means. More preferably, the container **110** is formed of polycarbonate or other plastic.

The container defines an interior chamber **112** and has first and second opposed ends **110A** and **110B**. A fitting **115** extends outwardly between the ends **110A**, **110B** and defines an inlet **116**, which fluidly communicates with the chamber **112**. A fitting **117** extends downwardly from the end **110A** and defines an outlet **118**, which fluidly communicates with the chamber **112**. The container **110** includes an end wall **114** on the end **110B**. Preferably, at least the end wall **114** of the container **110** is translucent or transparent. Optionally (not shown), the container **110** may be further provided with a vent port communicating with the chamber **112**. Preferably, the chamber **112** has a volume of between about 10 milliliters and 1 liter.

A sensor **120** is secured to the end wall **114** such that a surface **122** of the sensor **120** (**Figure 2**) is in fluid communication with the chamber **112**. The sensor **120** may be any suitable sensor for detecting or indicating a change in the chamber **112** caused by microbial growth. Suitable sensors and methods and materials for forming the same are disclosed in U.S. Patent No. 5,856,175 to Thorpe *et al.* and U.S. Patent No. 5,858,769 to DiGuiseppi *et al.*, the disclosures of which are hereby incorporated herein by reference in their entireties. The following sensors may be advantageously employed, but are not exhaustive or exclusive of the types of sensors which may be used in the invention.

As discussed below, a culturing medium may be added to the specimen. The culturing medium may be specially formulated to enhance the production of certain microbial metabolic products. These microbial metabolic products may be detected by the sensor **120**. The sensor **120** may comprise a solid composition or membrane (also referred to hereinbelow as an attachment or support medium), with an indicator medium immobilized on or within it. Suitable sensors for use as the sensor **120**, in various forms, include: a) a suspension of a solid particulate indicator medium immobilized within an immiscible fluid; b) a suspension of a solid particulate indicator medium immobilized within a polymer that is then

cured; c) a suspension of a liquid indicator medium impregnated or coated onto a solid support and immobilized within an immiscible fluid; d) a suspension of a liquid indicator medium impregnated or coated onto a solid support and immobilized within a polymer that is then cured; e) an emulsion of a liquid indicator medium in an immiscible fluid; f) an emulsion of a liquid indicator medium in a polymer that is then cured; and g) a membrane and an indicator medium, the indicator medium being selected for its ability to exhibit a detectable change when exposed to byproducts of an organism's metabolic activity.

The sensor **120** is preferably located flush against the inside surface of the container **110**. Preferably, the end wall **114** is transparent or translucent such that the indicator medium is visible from outside. The sensor **120** may be affixed to the container **110** to prevent cells, proteins, other solids or other opaque or colored components from getting between it and the container surface. In certain embodiments, the sensor **120** is separated from the specimen and its growth medium by a membrane, a viscous layer, or a solid layer that permits the passage of gas molecules but prevents passage of ions.

The nutritional components that make up a complex microbial medium influence the metabolic pathways used by microorganisms. Organic acids, bases and various gases are produced in proportions dependent on the nutrients available. These products also vary from species to species of microorganism. The presence of these products in the liquid medium can change its pH. The sensor **120** used in the invention may contain pH sensitive indicators that give a measurable change in response to a pH change in the environment. In the embodiment in which the pH sensor is covered by a gas-permeable, ion-impermeable membrane, the presence of gases that affect the pH of the indicator, such as CO_2 or ammonia, may be measured. Thus, microbial growth can be detected either by changes in pH of the liquid culture medium or by measurement of gases dissolved in the medium, both indications being caused by metabolic gaseous products produced by microorganisms. Carbon dioxide is a universal metabolite produced by all organisms and, therefore, is the preferred metabolite for detection of microbial

growth.

CO_2 and pH sensors as used for the sensor **120** may share two common components, a molecular species useful as a pH indicator and an attachment/support medium. The pH indicator can be attached either covalently or non-covalently to the support medium. Alternatively, the indicator can be encapsulated within a polymer matrix such as an indicator solution being emulsified within a polymer matrix prior to curing, or particles of solid indicator being suspended within a polymer matrix, which is then cured.

Also, the indicator can be attached to the solid support medium, for example, by soaking or impregnating the support medium with an indicator solution and then drying it. The support medium may be a membrane such as a nylon membrane. The dried, impregnated support medium can then be reduced to a fine powder, if needed, and blended with an immiscible fluid, such as polymer or a viscous fluid, forming a suspension sensor. In certain embodiments, the polymer can be cured. In other embodiments, a suspension sensor can be made by combining a solid indicator in particulate or granular-type form with an immiscible fluid, again, such as polymer, and then curing if needed.

To perform as a CO_2 sensor, the sensor **120** must be able to react with the byproducts of the microorganisms. The CO_2 sensor has a third component, a semi-permeable substance that completely separates the indicator membrane from the specimen and growth medium. The semi-permeable layer may be a separate membrane, alternatively, the cured polymer adjacent to the specimen and growth medium may form an integral semi-permeable membrane. These sensors may be affixed to the container **110** with an appropriate adhesive or as an indicator emulsified within a polymer matrix cured *in situ*.

A variety of different fluorescent and visible pH indicators can be used as the active molecular species in pH or CO_2 sensors. They should have acceptable dynamic pH ranges and wavelength changes that are readily detectable by front surface fluorescence or reflectance technologies.

Preferably, sensors for detecting pH changes in the culture medium

according to the invention exhibit a change in measurable properties such as fluorescence intensity or visible color at least over a pH range of about 5.0 to about 8.0. Preferably, indicators for the CO_2 sensors exhibit a change in measurable properties such as fluorescence intensity or visible color at least between about pH 13 and about 5, and more preferably between about pH 13 to about 9, in order to detect changes in CO_2 concentration.

Preferably, the pH indicators belong to the xanthene, phenolphthalein and phenolsulfonphthalein groups. Examples of these include fluorescein, coumarin, phenolphthalein, thymolphthalein, bromothymol blue, thymol blue, xylenol blue and α-naphthol benzein.

The attachment/support medium can be a substance such as cellulose, to which a pH indicator can be covalently attached using organic reactions. Non-covalent attachment of pH indicators can be achieved using ionic support materials, such as nylon membranes that have a positive or negative zeta potential. Other ionic support materials that can be used are positive or negatively charged ionic resins, such as diethylamino ethyl (DEAE) resin or DEAE cellulose. Pretreatment of the support material with a protein may be required if the indicator membrane is to be in direct contact with the microbial growth medium.

The pH indicator sensors directly detect pH changes due to the pH environment of the microbial growth medium. However, these sensors **120** can be made to selectively react to gases (*e.g.*, carbon dioxide, ammonia) in the liquid growth medium by covering them with a selectively semi-permeable composition or membrane, such as silicone, latex, teflon, or various plastics characterized by the capacity to selectively permit the diffusion of a gas while preventing the passage of ions. For sensors **120** comprising indicator encapsulated within a polymer matrix, such as emulsion or suspension sensors, the polymer forming the matrix can act as the semi-permeable barrier that permits the passage of gases but not ions.

In the emulsion sensor embodiments of encapsulated indicator, the CO_2 sensor is preferably comprised of four components. The first component is a

visual or fluorescent pH indicator, which is preferably reactive at least at the pH range between 6 and 10. Examples of indicators meeting these criteria are bromothymol blue, thymol blue, xylenol blue, phenolphthalein, coumarin, and fluorescein. The second component is sodium hydroxide or an equivalent base, which maintains an optimal pH environment for detection of CO_2 by the selected pH indicator. The third component is glycerol or an equivalent emulsifier, which can produce droplets of indicator solution emulsified within the uncured polymer. The fourth component is the uncured polymer such as silicone, which maintains a proper environment for the indicator. Any polymer can be used that does not affect the chemical activity of the indicator, either from its own chemical or physical properties or its requirements for curing, as long as it is permeable to gases but not ions, and does not have these properties altered when subjected to sterilization. Other silicone polymers that are also satisfactory are those that are cured by high temperature, by catalytic activity, or by ultraviolet vulcanization. An emulsion is prepared from the four components and the polymer is cured to form a semipermeable matrix around the droplets of pH indicator, which permits selective diffusion of CO_2 and other gases from the liquid microbial growth medium, resulting in a measurable change in the indicator. The sensor can be prepared separately, such as in a mold, cured, and then attached to the container **110** with an appropriate adhesive, such as a silicone adhesive. Alternatively, and preferably, the sensor **120** is formed on the end wall **114** of the container **110** and cured *in situ*.

Similarly, the CO_2 sensor can be manufactured as a suspension, incorporating many of the same elements as above. Generally, a suspension is defined as a system in which very small particles are more or less uniformly dispersed in a liquid medium. If the particles are small enough to pass through filter membranes, the system is a colloidal suspension. If the particles are larger than colloidal dimensions they tend to precipitate, if heavier than the suspending medium, and to agglomerate and rise to the surface, if lighter. (Hawley's Condensed Chemical Dictionary, edited by N. Sax and R. Lewis, Sr., 11th edition,

1987, N.Y., N.Y.)

A granular or particulate-type of indicator medium can be blended or mixed with a suitable immiscible fluid, whereby the result is a suspension of the solid indicator medium in the fluid. If a polymer is used as the fluid, it can be cured after forming the suspension to produce a solid suspension sensor.

By adding a support medium to an indicator solution, another type of suspension sensor can be produced. In this embodiment, the indicator medium is a solution, such as a suitable soluble indicator medium dissolved in a NaOH solution, which is attached to a solid support medium, for example, by impregnating or coating the solid support medium. The indicator solid support medium is then dried, and can be reduced to a fine powder, for example by cutting or grinding, or used as is. The impregnated or coated support is blended with an immiscible liquid, such as a polymer, as above, forming a suspension. The suspension sensor can be used in the same way as the emulsion sensor above. As with the emulsion sensor, when the suspension sensor involves the use of a polymer that may need to be cured, it is preferable to form the suspension sensor directly in the container **110** and then cure it *in situ*. If so desired, a semi-permeable material may be placed over the suspension sensor to separate the sensor from the liquid or solid contents of the container. For example, an overlay (not shown) of a semi-permeable polymer or immiscible liquid may be placed over the suspension sensor. Preferably, the overlay reflects light that passes through the sensor **120** and also protects the sensor **120** from direct contact with the liquid or solid contents of the container.

An exemplary sensor **120** may be formed of xylenol blue, NaOH, borate buffer and Triton X-100.

As a further alternative, the sensor **120** may be an O_2 sensitive fluorescent sensor. As microbes in the chamber **112** grow, they consume O_2 thereby reducing the amount of O_2 at the sensor **120**. The fluorescence of the sensor, which is quenched by O_2, increases as the O_2 concentration decreases. Microbial growth can thereby be detected by the increase in fluorescence. For example, the sensor

120 may be a sensor as disclosed in U.S. Patent No. 5,998,517 to Gentle *et al.* and U.S. Patent No. 5,567,598 to Stitt *et al.*

Another type of sensor that may be used for the sensor **120** includes a piezoelectric apparatus (not shown), such as a piezoelectric strip, that is attached to the container **110**. The signal from the piezoelectric apparatus can be automatically zeroed when the container **110** and its contents reach a given or selected temperature (*e.g.*, an incubation temperature). The piezoelectric apparatus can be constructed and/or mounted such that it is distorted by the pressure of the metabolic products produced by microorganisms. The electrical signals generated from this distortion can be measured to monitor the biological activity inside the container **110**.

Referring again to **Figures 1** and **2**, a collection filter **130** is mounted adjacent the end **110A** of the container **100** and between the inlet **116** and the outlet **118**. In certain embodiments, the filter **130** is constructed and mounted such that substantially all fluid passing from the inlet **116** to the outlet **118** passes through the filter **130**. As shown, the filter **130** is adapted to collect all or a substantial portion of potential or anticipated microorganisms from a specimen while allowing the carrier fluid of the specimen to pass through the filter **130**. In **Figure 1**, the filter extends across the entire cross-sectional area of the chamber. Preferably, the filter **130** is a microporous filter configured to capture microorganisms therein while passing soluble components of the specimen, wash fluid or culturing medium. The microporous filter may be hydrophobic or hydrophilic. Preferably, the pores of the microporous filter are no larger than 0.4 micron, and, more preferably, are between about 0.2 and 0.4 micron. Suitable filter membranes for the filter **130** include product no. GSWP 0500 (hydrophilic, cellulose-based, for aqueous solutions) and product no. FGCP 04700 (hydrophobic, Teflon ™-based, for solvent solutions), each available from Millipore Corporation of Bedford, Massachusetts.

With reference to **Figures 3-7** and the flow chart of **Figure 8**, the device **100** may be used in the following manner to filter and detect the growth of

microorganisms from a specimen. Initially, the device **100** (including the filter **130** and the sensor **120**) as well as the tubing **142**, **152**, **162** and **172** described below may be sterilized, for example, using an autoclave, chemical disinfectant or radiation. As shown in **Figure 3**, a supply **140** of the fluid (typically liquid) specimen is connected to the fitting **115** of the device **100** by suitable tubing **142**. The specimen may be, for example but not limited to, potable water, beverage or food products, pharmaceuticals and their production intermediates, or parenteral fluids.

With further reference to **Figure 3**, a waste receptacle **150** is connected to the fitting **117** by suitable tubing **152**. The waste receptacle **150** is in turn operatively connected to a fluid flow source such as a vacuum source **154** which may be a pump. Alternatively, a pump (*e.g.*, a peristaltic pump) may be provided at the tubing **142**. Other flow sources may also be used. For example, the specimen may be gravity fed. The vacuum source **154** is operated to create a vacuum at the inlet **116** (**Figure 2**) of the device **100**. The specimen is thereby drawn, sequentially, from the supply **140**, through the tubing **142**, through the inlet **116**, through the filter **130**, through the outlet **118** (**Figure 2**), through the tubing **152** and into the waste receptacle **150** (Block **192** of **Figure 8**). As the specimen passes through the filter **130**, microorganisms (if present) in the specimen are collected on the filter **130**, captured or trapped (*i.e.*, inhibited or prevented) from passing with the remainder of the specimen to the waste receptacle **150**. These captured microorganisms constitute a sample which may be used to evaluate for the presence of microbes in the specimen supply **140**. Advantageously, the concentration of microorganisms in the sample may be substantially increased as compared to the concentration of the microorganisms in the specimen so that changes caused by microbial growth will be detected earlier and thereby more effectively. Preferably, substantially all of the specimen not captured in the filter **130** is drawn out of the chamber **112**. Typically, the volume of the filtered specimen may be in the range of between about 100 milliliters and 10 liters.

With reference to **Figure 4**, an optional wash step may be conducted. More particularly, a wash fluid supply **160** may be connected to the fitting **115** by suitable (preferably sterile) tubing **115**. The wash fluid is drawn or directed sequentially through the inlet **116**, the filter **130** and the outlet **118** and into the waste receptacle **150** by the vacuum source **154** (Block **193**). The wash step may be used, for example, to wash preservatives or other microbe growth inhibitors from the container **110**, the filter **130** and the captured sample. Suitable wash fluids may include, for example, growth media, buffered salt solutions, detergents or emulsifiers and are sterilized prior to introduction into the chamber **112**.

Thereafter, and preferably after the wash fluid is drained from the chamber **112** (not shown in **Figures 3-5**), a supply **170** of culturing medium or broth is connected to the fitting **115** by suitable tubing **172** (**Figure 5**). Preferably, the tubing **172** and the culturing medium are sterile. Preferably, the device **100** is inverted as shown in **Figure 5**. Thereafter, the culturing medium is fed, for example, by gravity feed, from the supply **170**, through the tubing **172**, through the inlet **116** (**Figure 2**) and into the chamber **112** (Block **194** of **Figure 8**). The outlet **118** (**Figure 2**) serves as a vent for the air in the chamber **112** displaced by the culturing medium. The culturing medium is preferably sterilized prior to introduction to the chamber **112**. A one-way valve may be used to inhibit the entry of fluids or air as needed (not shown).

With reference to **Figure 6**, a cap **175** (preferably a screw cap) may be placed over the fitting **117**. A suitable clamp **174** is clamped over the tubing **172**. The tubing **172** is then cut above the clamp **174** such that a portion **172A** of the tubing **172** remains attached to the fitting **115**. In this manner, the tubing **172A** and the clamp **174** form a secure and sterile closure to the inlet **116**. The device **100**, the tubing **172A**, the clamp **174**, the contained culturing medium **171** and the captured microorganism sample together form a sample assembly **101** (**Figure 6**). Other closure or sealing techniques can also be used such as adhesives, heat to close the tube, tie wraps and the like.

The sample assembly **101**, or a plurality of sample assemblies **101**, may then be placed in a suitable measuring apparatus **180** (Block **195** of **Figure 8**). The measuring apparatus may be an automated apparatus as described in U.S. Patent No. 5,858,769 to DiGuiseppi *et al.* or as described in U.S. Patent No. 5,164,796 to DiGuiseppi *et al.*, the disclosures of which are hereby incorporated herein by reference in their entireties. The sample assembly **101** may be inserted into a slot **184** in a platform **182** for evaluation by a detector assembly (generally designated **181**). The detector assembly **181** includes a photo-emitter **186** and a photodetector **188**, each of which is operatively connected to an analysis apparatus **189**. The photo-emitter **186** directs light onto the sensor **120** and the light is reflected back to the photodetector **188**. The analysis apparatus **189** evaluates signals from the photodetector, for example, in the manner described in U.S. Patent No. 5,164,796. The microorganism analysis results can be automated and results generated without requiring human evaluation.

In the foregoing manner, the measuring apparatus **180** detects changes in the sensor **120** and thereby measures microbial growth in the chamber **112** (Block **196**). For example, the measuring apparatus **180** may detect changes in the color or fluorescence of the sensor through the transparent end wall **114**, such color or fluorescence changes being caused by pH changes, the generation of CO_2 and/or the consumption of O_2 in the chamber **112** from microbial growth. For example, the measuring apparatus **180** may include instruments as described in U.S. Patent Application Serial Nos. 07/322,874, filed on April 3, 1989 (now abandoned), and 07/351,476, filed on May 15, 1989 (now abandoned), U.S. Patent No. 4,945,060 and U.S. Patent No. 5,856,175, which are incorporated herein by reference. The apparatus **180** may include a visible light reflectometer that monitors the color change of the sensor. Solid state illuminators and detectors may be used. Incandescent and arc lamp sources of illumination may also be used in conjunction with mirrors, lenses, optical fibers, and other means of directing the light to the sensor. In order to allow continuous monitoring of all samples, it is preferred to have a detector for each sample. The outputs of the various detectors may be

compiled by a computer, and curves characteristic of the quantity and rate of change of pH or CO_2 or O_2 concentration of various samples may be generated. The computer may also perform the necessary analysis to evaluate the characteristics developed and to determine the presence or absence of developing microbial cultures. The apparatus **180** and/or additional apparatus may heat and/or agitate the device **100** to incubate or otherwise promote growth of the sample microorganisms.

With reference to **Figure 9**, an integrated filtration and detection device **200** according to further embodiments of the present invention is shown therein. The device **200** includes a container body **210** defining an interior chamber **212**. The container body **210** has an end wall **214** and defines an opening **212A** communicating with the chamber **212**. Preferably, the end wall **214** is transparent.

An end cap **219** is disposed over the opening **212A**. An O-ring **211**, gasket, adhesive or other sealant may be used to provide a seal between the cap **219** and the container body **210**. A second fitting **215** extends through the cap **219** and defines an inlet **216**. A second fitting **217** extends through the cap **219** and defines an outlet **218**.

A radial flow filter **230** is secured to the underside of the cap **219**. Preferably, the filter **230** is a microporous filter. The filter **230** is preferably constructed and mounted such that substantially all fluid passing from the inlet **216** to the outlet **218** passes through the filter medium of the filter **230**. A second filter **232** can be positioned between the filter **230** and the outlet **218**. Preferably, the second filter **232** is also a microporous filter.

A sensor **220** may be secured to the end wall **214** (or other desired externally visible location). The sensor **220** may be any of the sensors and may be mounted in any of the ways as described above with regard to the sensor **120**.

The device **200** may be used in substantially the same manner as the device **100** with connections being made with the fittings **215** and **217** in place of the fittings **115** and **117**, respectively. It may be desirable to invert the device **100** during the steps of filtering the specimen and introducing the wash fluid. Tubing

272 corresponding to the tubing **172** may be cut and clamped or secured in the same manners described above to provide a secure and sterile closure. The filter **232** serves to prevent or inhibit entry of contaminants into the chamber **212** through the outlet **218**. Additionally, a cap may be placed over the fitting **215** and/or the fitting **217**. Also, either or both of the fittings **215**, **217** may be provided with a one-way valve.

As a further alternative, the sensor **220** may be secured to or may be made part of the cap **219** (not shown). The cap or a section thereof may be transparent so that, in the case of a sensor of the type that changes color, the changes in the color of the sensor **220** may be evaluated through the end cap **219**. The end cap **219** may be made of a material, such as a polymer, which contains encapsulated indicator micelles.

The sensors **120**, **220** may be relocated in the container **110** or container body **210**. In the case where color changing sensors are employed, each container should include at least a transparent (or translucent) section adjacent the sensor **120**, **220**. Alternatively, the sensors **120**, **220** may each be formed (*e.g.*, molded) as an integral part of the respective container **110** or container body **210**. The sensors **120**, **220** may also be placed outside the container, as long as sealed (from the environment) fluid communication means are provided for the metabolic products of the microorganisms or the growth medium containing the specimen to react with the sensor.

The foregoing devices **100**, **200** and methods and variations thereof as described above provide a number of advantages. The sample may be collected, incubated and evaluated without breaking the sterility barrier. The devices and methods serve to combine certain steps of preparing a sample and detecting microbial growth in the sample. The devices and methods eliminate or reduce handling of various components, thereby providing enhanced convenience and greater security against inadvertent contamination during testing and evaluation. In particular, the sample, the filter, the sensor and adjacent interior portions of the container are protected from contamination, thereby reducing or eliminating the

risk of a false positive caused by microorganisms not introduced from the original specimen.

The foregoing is illustrative of the present invention and is not to be construed as limiting thereof. Although a few exemplary embodiments of this invention have been described, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiments without materially departing from the novel teachings and advantages of this invention. Accordingly, all such modifications are intended to be included within the scope of this invention as defined in the claims. Therefore, it is to be understood that the foregoing is illustrative of the present invention and is not to be construed as limited to the specific embodiments disclosed, and that modifications to the disclosed embodiments, as well as other embodiments, are intended to be included within the scope of the appended claims. The invention is defined by the following claims, with equivalents of the claims to be included therein.